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Roads as a driver of changes in the bird community and disruptors of Ecosystem Services provision

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Roads as a driver of changes in the bird community and disruptors of Ecosystem Services provision.

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1. Resumo

O montado é considerado um ecossistema vital devido ao seu valor económico e importância para a biodiversidade. No entanto, este ecossistema tem vindo a sofrer um declínio nos últimos anos, com uma elevada ocorrência de pragas de insetos nas zonas de sobreiro. A rede rodoviária tem impactos ambientais negativos diretos e indiretos na fauna, principalmente nas aves, que atuam como agente de controle biológico de pragas e previnem possíveis surtos. Embora a relação entre estradas e aves, bem como as relações presa-predador entre aves e pragas tenham sido alvo de vários estudos, pouco se sabe sobre como as estradas influenciam a eficiência do controle biológico de pragas efetuado pelas aves e como esse serviço de ecossistema pode ser mantido ou mesmo melhorado para compensar os efeitos negativos das estradas. Consequentemente, o nosso objetivo foi avaliar se a proximidade da estrada afeta a estrutura das relações presa-predador do montado. Os locais de amostragem foram selecionados nas proximidade das principais estradas pavimentadas (N119, N10 e A13). Os censos de aves durante o inverno foram realizados em vinte e cinco locais, cada um com três locais de amostragem com distância crescente à estrada para avaliar as relações entre a abundância de aves, a abundância de pragas de sobreiro e sua variação com a proximidade à estrada. Devido às restrições logísticas da pandemia COVID-19 (Confinamento), houve uma redução no período de amostragem e, consequentemente, uma redução do número de locais de amostragem e distância entre os pontos de amostragem. Os censos de pragas e aves durante a primavera foram realizados em onze locais dos vinte e cinco locais previamente amostrados, cada um com dois pontos de amostragem com distância crescentes à estrada. Adicionalmente, nos mesmos locais recolhemos dados sobre o nível de infeção de duas espécies de cobrilha, *Coroebus florentinus* e *Coroebus undatus*, uma vez que estas espécies são consideradas pragas de elevada importância devido aos danos que causam às árvores. Durante o período de amostragem foram detetadas 41 espécies de aves e 8 grupos de pragas de sobreiro. Os resultados deste estudo revelaram uma diminuição da abundância e riqueza de aves com a proximidade à estrada. O padrão oposto foi encontrado para as pragas, a abundância de pragas aumentou com a proximidade à estrada, com quatro espécies de praga de sobreiro a apresentarem uma abundância maior nas árvores próximas da estrada. *Coroebus florentinus* e *Coroebus undatus* apresentaram uma diminuição nos níveis de infeção com o aumento da distância à estrada. Duas espécies de aves apresentaram menor presença perto das estradas, enquanto apenas uma espécie apresentou menor presença com o aumento da distância à estrada. A abundância de pragas e o nível de infeção por *Coroebus florentinus* e *Coroebus undatus* foi maior na estrada N10, onde a abundância de aves foi menor. Os resultados mostraram que a abundância de aves diminui com a proximidade da estrada, enquanto a abundância de pragas de sobreiro aumenta, sugerindo uma relação causa-efeito entre os dois grupos, mediada pelos impactos da estrada. Estes resultados fornecem a primeira evidência de que as estradas podem afetar a provisão de serviços de ecossistema, particularmente por interromperem o controle de pragas realizado pelas aves no montado, e antecipamos que tal impacto se traduza em perdas importantes para a economia da cortiça.

Palavras-chave: Ecologia de estradas, Controle biológico de pragas, Relações presa-predador, Conservação

2. Abstract

The Mediterranean oak woodlands are a vital ecosystem due to their economic value and importance for biodiversity. However, this ecosystem has shown a decline in recent years, with a high occurrence of insect pests in cork oak areas. The road network has direct and indirect negative environmental impacts on wildlife, particularly on the bird population, that act as biological pest control agents and prevent possible outbreaks. Although the relationship between roads and birds, as well as the predator-prey relationships between pests and birds has been widely examined, little is known about how roads influence the efficiency of pest control provided by birds and how this ecosystem service can be maintained or even improved to compensate for roads negative effects. Accordingly, we aimed to assess if road proximity affects the assembly and structure of the predator-prey relationship of Mediterranean oak woodlands. Sampling sites were established in the proximity of the main paved roads (N119, N10 and A13). Bird surveys during winter were performed at twenty-five sampling sites, each one with three sampling points at increasing distances to the road, to assess the relationships between abundance of birds, abundance of cork oak pests and their variation with road proximity. Due to logistic constrains of COVID-19 pandemic (lockdown), there was a reduction in sampling period, and consequently a reduction of the number of sampling site and distance between sampling points. Pest and bird surveys during spring were performed at eleven sites of the twenty-five sites previously sampled, each one with two sampling points at increasing distances to the road. Furthermore, in the same sampling points we collected data regarding the degree of infection of two species of buprestids, *Coroebus florentinus* and *Coroebus undatus*, because these species are considered significant pests due to the damage they cause to the tree. During the sampling period 41 bird species were detected and 8 groups of cork oak pests. As result, bird abundance and richness decreased with proximity to roads. The opposite pattern was found for pests, pest abundance increased with proximity to the road, with four species of cork oak pest showing a higher abundance in trees close to roads. Both *Coroebus florentinus* and *Coroebus undatus* showed a decrease in the degrees of infection as the distance to the road increase. Two bird species assessed showed significant lower presence near the roads, whereas only one displayed lower presence as the distance to the road increased. Furthermore, pest abundance and the degree of infection by *Coroebus florentinus* and *Coroebus undatus* was higher in road N10, where bird abundance was lower. The results showed bird abundance decreases with road proximity, whereas the abundance of cork oak pest increase, suggesting a cause-effect relation between the two groups, mediated by road effects. These results provide the first evidence that roads may affect the provision of ecosystem services, particularly by disrupting the pest control by birds in cork-oak systems. We anticipate that such impact may translate into important losses to cork economy.

Keywords: Road ecology, Biological pest control, Prey-predator relations, Conservation

3. Introduction

Nowadays infrastructures such as roads, telephone or electricity cables, highways and gas lines are common features since they are necessary for human activities and development, such as transportation of goods and services and as a way of connection. However, theses infrastructures have direct and indirect negative environmental impacts on ecosystems and biodiversity worldwide (Benítez-López et al., 2010; Møller et al., 2011; Morelli et al., 2015; Van der Ree et al., 2015), such as habitat degradation, habitat loss, chemical pollution, wildlife mortality, barrier effect and population fragmentation (Van der Zande et al., 1980; Kuitunen et al., 1998; Rheindt, 2003; Rytwinski and Fahrig, 2015; Van der Ree et al., 2015). The road network is a well-known problem to wildlife and may become even bigger, as this network is still expanding and roadless areas are becoming rare (Selva et al., 2015; Van der Ree et al., 2015). Roadless areas are important for preserving biodiversity and providing ecosystem services (Selva et al., 2015; Ibisch et al., 2016). However, some countries in the world already have such an extensive road network that these areas are few and small, more than half are less than 1 km^2 in size and only 7% are over 100 km^2 (Ibisch et al., 2016). Portugal alone contains nearly 45 000 km of paved roads (Infraestruturas de Portugal, 2016), and a road density of 0.5 km/km^2, which can have a significant effect in species' occurrence. The impacts of roads in wildlife populations have been extensive documented and there are various published studies demonstrating that roads represent a threat to biodiversity (Erritzoe et al., 2003; Rheindt, 2003; L. Quinn et al., 2006; Fahrig and Rytwinski, 2009; Benítez-López et al., 2010; Møller et al., 2011; Cooke et al., 2020b).

Birds are well documented in road ecology literature, with several studies showing a reduction in bird populations near roads (e.g., Fahrig and Rytwinski, 2009; Benítez-López et al., 2010; Summers et al., 2011; Cooke et al., 2020b), especially near high-traffic roads (e.g., Kuitunen et al., 1998; Rheindt, 2003; Fahrig and Rytwinski, 2009; Summers et al., 2011). These declines can be due to disturbance by traffic emissions, such as noise, lights and chemical emissions. Traffic noise is a major cause of bird abundance decline as it can interfere with birds acoustic communication by which they depend on to survive (Fahrig and Rytwinski, 2009; McClure et al., 2013; Rytwinski and Fahrig, 2015; Ware et al., 2015). Even though, Slabbekoorn and Ripmeester (2008) acknowledge that some species can adjust their singing to overcome the presence of noise, species without this ability cannot live in a noisy environment (Summers et al., 2011; McClure et al., 2013). Moreover, noise pollution affects prey-predator relations as in the presence of noise birds increase their vigilance which can compromise their foraging activity and consequently affect their health (L. Quinn et al., 2006; McClure et al., 2013; Ware et al., 2015), since bird species that are exposed to noise feature a decrease in body condition and stopover efficiency, their ability to gain body condition over time (Ware et al., 2015). Light pollution can also disturb birds through cars headlights (Summers et al., 2011) and public illumination in road surroundings (Dominoni et al., 2013). These light sources can affect bird navigational abilities and interfere with the timing of their circannual events, such as migration, breeding and physiological changes (Dominoni et al., 2013). Chemical emissions and contaminants from cars can lead to air, water, and soil pollution (Van der Zande et al., 1980) which affect birds directly and indirectly by killing their food resources (Summers et al., 2011), and overall reduce habitat quality (Rytwinski and Fahrig, 2015). Birds can also be affected through collision with vehicles. In Western Palearctic, 5 to10% of bird mortality is due to animal-vehicle collisions (Møller et al., 2011), with passerines being among the more susceptible to traffic (Erritzoe et al., 2003). These numbers suggest that most collisions may involve species abundant in anthropogenic habitats, such as the house sparrows *Passer domesticus* (Erritzoe et al., 2003; Møller et al., 2011; Cooke et al., 2020b), or species that benefit from resources provided by roads, such as some raptor and corvids (Erritzoe et al., 2003; Fahrig and Rytwinski, 2009; Benítez-López et al., 2010; Morelli et al., 2014; Rytwinski and Fahrig, 2015; Cooke et al., 2020b). These resources can be food, water, hunting perches (telephone or electricity cables and poles), or nest building material (Erritzoe et al., 2003; Morelli et al., 2014; Rytwinski and Fahrig, 2015). Roads can also create edge habitat along the roads and introduce new features to the habitat that provide benefits, such as hedges and ditches (Morelli et al., 2014; Cooke et al., 2020b). Although roads have advantages, for birds to access these resources they must be exposed to a risk of collision with impacts on their health and breeding success. So, ultimately, roads have a significant effect in species abundance and richness which may disrupt the ecosystem functioning and affect ecosystem services (Selva et al., 2015; Van der Ree et al., 2015).

Birds provide various ecosystem services, such as pollination, decomposition, seed transportation, biological pest control, and nutrient recycling. Birds can act as biological pest control agents and prevent possible outbreaks of pests by declining or maintaining a low abundance of insects and other pests. Through the control of pest populations, birds can decrease the impacts on the resource the pest consumes (Whelan et al. 2008) and, thus, reduce economic loss (Mols and Visser, 2007; Bereczki et al. 2014) and maintain the health of the ecosystem (Şekercioğlu et al., 2004). Due to their predatory behavior regarding various types of pests, birds can play an important role as pest control agents in different ecosystems (Bereczki et al., 2014).

Insects can be ecosystem key elements as pollinators and source of nutrients. However, some species have cyclic outbreaks that cause extensive devastation and, in extreme cases, decline of the ecosystem (Pimentel and Nilsson, 2007; Barbaro and Battisti, 2011; Ceia and Ramos, 2016). Many studies have demonstrated that, under certain conditions and abundances, insects can become pests (Boyd et al., 2013) by increasing tree vulnerability and reducing crop yields. Agricultural pests, especially herbivorous insects, can cause an annual loss of approximately 7.7% in production equivalent to 25 million tons of food, fiber, and biofuels (Oliveira et al., 2014). During several years, the solution to this problem was the use of insecticides due to their efficiency and profitability of production but these insecticides also affect the population of natural enemies which, in the long term, can lead to more losses of crops. Some of these pests can develop resistance to insecticide and cause economic impact to crop fields by damaging flowers and fruits (Solomon et al., 2010). Furthermore, in the future, this problem may present a tendency to intensify since several pests are developing resistance to synthetic insecticides, mainly because of their short generation times and their coevolution with plant toxins (Pimentel and Lehman, 1993). Also, changes in public opinion concerning insecticides and their impacts on the environment and human health is leading to the creation of new legislation regarding their use (Solomon et al., 2010). For these reasons, biological control through birds is becoming a possible and viable alternative since it has no impacts and is a process that already occurs naturally in the ecosystem.

There are several examples demonstrating the benefits of birds as pest control agents. Apple and pear orchards can have an abundant and diverse arthropod fauna where 25% of them can act as pests, such as codling moth *Cydia pomonella* and winter moth *Operophthera brumata* (Solomon et al., 2010). However, apple orchards with populations of Great tit *Parus major* have a 50% reduction in damage to apples, even under high caterpillar density (Mols and Visser, 2007). Bark beetles, such as *Dendroctonus* species, are considered pests in coniferous forests (Franceschi et al. 2005; Fayt et al., 2015). These beetles create galleries in the tree bark and introduce a pathogenic fungus that reduces the defences of the tree, by destroying the phloem and cambium, creating the appropriate environment for the laying and growth of the larvae (Franceschi et al. 2005). As bark beetles have periodic outbreaks, they can cause the death of millions of healthy trees each year (Franceschi et al. 2005; Fayt et al., 2015). Woodpeckers can act as pest control agents by reducing bark beetles populations up to 98%, even in beetle populations with epidemic degrees (Fayt et al., 2005). The pine processionary moth *Thaumetopoea pityocampa* is an important pine defoliator and one of the most dangerous pests in pine forest of Portugal and other Mediterranean regions, due to the negative impacts caused on pine trees such as total loss of leaves (Arnaldo and Torres, 2005; Pimentel and Nilsson, 2007), and the repercussions in human and animal health, made by their bristle which cause urticaria and allergy. In

1997, was recorded for the first time a temporally life cycle shift in a Portuguese population of pine processionary moth, with larval development occurring in summer rather than winter (Pimentel and Nilsson, 2007). This change happened due to climate fluctuations and can disrupt interactions between consumers and resources by affecting their distribution, phenology, or behavior (Parmesan and Yohe, 2003; Pimentel et al., 2011). Fortunately, this change made eggs and moths more available for birds, such as the Great tit, during the breeding season, particularly for their second clutch (Pimentel and Nilsson., 2007). This allowed the control of the population of pine processionary moth despite the life cycle shift and prevented a possible outbreak.

The Mediterranean oak woodlands are a vital ecosystem due to their economic value and importance for biodiversity (Bugalho et al., 2011; Pereira et al., 2015).). This ecosystem occurs in the western Mediterranean basin (Bugalho et al., 2011) and its characterized by forested areas of anthropogenic origin dominated by two species of evergreen Mediterranean oaks, cork oak *Quercus suber* and holm oak *Quercus rotundifolia* (Pinto-Correia and Mascarenhas, 1999; Godinho and Rabaça, 2011; Pinto-Correia et al., 2011; Pereira et al., 2015). Since Mediterranean oak woodlands have occasional or frequent human intervention can be portrayed as a combination of agricultural, pastoral, hunting activity and forest uses from where humans can get multiple goods and services (Bugalho et al., 2011; Pinto-Correia et al., 2011; Pereira et al., 2015). In addition to its economic value, Mediterranean oak woodlands are considered an important habitat for the preservation of insects, migratory and resident birds, and endangered bird species (Bugalho et al., 2011; Leal et al. 2011; Pereira et al., 2015). Thus, it is important to preserve this ecosystem because of its biological diversity, for it is necessary to have good management practices (Leal et al. 2011) that improve wildlife and cork productivity. Since a poor or non-existent management and abandonment of Mediterranean oak woodlands can put the ecosystem at risk, as it requires active management and continuous use by humans to guarantee its existence (Bugalho et al., 2011; Pereira et al., 2015). In addition, an over exploration of some services, as livestock production and cork extraction, can cause degradation of the ecosystem (Pinto-Correia and Mascarenhas, 1999). The replacement of Iberian black swine by heavy cattle brads in this ecosystem causes a direct damage to the soil and the root system (Pinto-Correia and Mascarenhas, 1999), compromising the well-being of tree. Also, an overexploitation of the tree, by pruning, thinning and cork extraction, reduces the foraging substrate and weakens the trees making it more vulnerable to pests (Pinto-Correia et al., 2011; Leal et al. 2013).

In Mediterranean oak woodlands, there are 92 species of herbivorous insects that attack cork oaks frequently or occasionally (Ferreira and Ferreira, 1986) and 20 of them are considered pest, since they cause regular damage and substantial economic losses (Ceia and Ramos, 2016). Defoliators and borers are the ones that cause the most direct damage to the trees (Boyd et al, 2013). Defoliators attack the leaves and reduce the growth of the tree. Moreover, extensive defoliation can reduce the cork quality or potentiate the attack of other pests. For instance, gypsy moth *Lymantria dispar* is a major defoliator due to its recurrent outbreaks and larvae's voracity that can cause completely defoliation in one year (Tiberi et al., 2016). Bark-/wood-boring insects are considered significant pests due to the damage they cause (Tiberi et al., 2016). These insects attack the trunk and branches of the tree causing its death, especially if it is already weakened or damaged (Sousa et al., 2007; Pereira et al., 2015). For example, larvae activity of *Coroebus undatus* creates galleries under the trunk bark, which makes stripping difficult, and in extreme conditions impossible to perform (Pereira et al., 2015). Although the causes of decline in Mediterranean oak woodlands are multifactorial, the first evidence of cork oak decline due to pests occur at the end of the 19^{th} century (Almeida, 1898; Câmara-Pestana, 1898), the trees began to show symptoms of infection, such as trunk cankers, wounds, reduced branch growth, necrosis in the root cortex, defoliation and transparency of the crown and finally death (Branco and Ramos, 2009). Many of these symptoms can still be seen today. Throughout the 20th century, an evolution in insect populations was observed, caused by the imbalance of this ecosystem, leading to the appearance of new species and an increase in abundance (Sousa et al., 2007). In addition, insect pests with occasional outbreaks, which previously did not significantly affect the ecosystem, start to increase the instability of cork oak trees and compromised its regeneration (Neves, 1950; Ceia and Ramos, 2016). Furthermore, a study regarding canopy insects found out that insect pests have a high occurrence in cork oak area and an important role in the decline of Mediterranean oak woodlands (Boyd et al., 2013; Pereira et al., 2019) which brought to the realization that it is necessary to take measures to prevent it (Pereira et al., 2019).

In Portugal, there are more than 300 species of birds and 35% of this species can be found in Mediterranean oak woodlands (Pereira et al., 2015). The predominant guild of birds in this ecosystem is insectivorous birds (Godinho and Rabaça, 2011; Leal et al. 2011; Pereira et al., 2015) which can prey on all the life cycle states of insects. In Mediterranean oak woodlands, the sprouting of new leaves occurs in spring, which coincides with the development of herbivorous insects. Several birds, such as woodpeckers, tits and Eurasian nuthatch *Sitta europaea*, also have their breeding season during spring which increases the demand for food (Pereira et al., 2015). Previous studies showed that, during spring, insectivorous birds can reduce up to 90% the populations of pests (Parry et al., 1997). So, a possible solution to counteract the current decline of Mediterranean oak woodlands would be measures to maintained or even improved population of insectivorous birds since these birds act as natural regulators of insect populations.

Although the relationship between roads and birds (Kuitunen et al., 1998; Rheindt, 2003; Fahrig and Rytwinski, 2009; Benítez-López et al., 2010 Summers et al., 2011; Cooke et al., 2020b), as well as the predation of pests by birds has been widely examined (Parry et al., 1997; Sousa et al., 2007; Pimentel and Nilsson, 2007; Barbaro and Battisti, 2011; Boyd et al., 2013; Ceia and Ramos, 2016), little is known about how roads influence the efficiency of pest control provided by birds and how this ecosystem service can be maintained or even improved to compensate for roads negative effects. Birds can be used as indicators of the ecological status of an ecosystem, through the application of bird surveys (Pereira et al., 2015). Bird surveys are performed using one of these methods, point counts or line transects, and the choice of method depends upon several factors. Point counts consist in walking to a previously determined spot and record all bird contacts, seen or heard, during a fix period - normally 5 to 10 minutes – before moving to the next point. This method allows the observer to fully concentrate on the birds and habitat and gives more time to identify each contact. Also, point counts can be used to relate bird occurrence to habitat features and it is a good method to detect cryptic and skulking species. Line transects consist in continually walking a pre-determined distance and record all bird contact in both sides of the track. This method permits the collection of abundance and density data and allows the observer to cover more ground with less chances of double counting the birds. Additionally, line transects do not depend on the territorial behavior of species and can be used to detect species that are more conspicuous and mobile. In comparison to point counts, errors in distance estimation in line transects are less serious. For example, if the target of the study are species easy to identify but with low densities and high mobility it is better to use line transects. However, if the target of the study is a group of bird species, such as insectivorous birds, or small and conspicuous species in fine-grained habitats point-counts are the best method to use (Bibby et al. 1992). Regarding pests, there are several sampling techniques for studying insect pest in Mediterranean oak woodlands. The choice of techniques may depend on specie or group of species, the insect’s stage of life or the area of the tree affected by the pest. There are two main techniques for assessing the degree of infection by defoliators, the collection of branches for monitoring leaves and the use of traps. Leaf monitoring is a more widespread technique because it is based on the abundance of adults, larvae, or signs of presence, according to the specie or group of species under study. For this reason, this method is used to evaluate several species in simultaneously. There 3 types of traps, luminous traps, pheromone traps and glue traps. However, when using traps, the best technique is to combine different types of traps to complement each other. For example, the combination of glue traps and pheromones so that when the insect is attracted to the trap due to the pheromones, it stays glued. Also, pheromone traps can only be used to capture adults from the order Lepidoptera. There are six techniques for studying branches and trunk borers: counting dry branches, collecting ants from the trunks, counting postures on the trunk, collecting defoliating caterpillars, measuring galleries in cork oaks after stripping and counting of holes in the trunks resulting from insect activity. Therefore, the choice of method depends on the species to be evaluated. Counting dry branches is used to assess the degree of infection by *Coroebus florentinus*, since the activity of this specie larvae interrupts the flow of sap causing the death of the branch. To evaluate the degree of infection by *Coroebus undatus*, the galleries present in the tree trunk are measured. Also, both these methods can be performed at the same time as the collection of branches (Pereira et al., 2015).

The goal of this study was to assess if road proximity affects the assembly and structure of the predator and prey communities of Mediterranean oak woodlands. We aimed to assess the relationships between abundance of birds, abundance of oak pests and their variation with road proximity. We tested the following hypothesis: (h1) the proximity to the road has a negative effect on bird abundance and (h2) due to a decrease in bird abundance an indirect positive effect on pest abundance. Through the examination of sampling sites at increasing distances from paved roads, we expected that bird abundance would be lower near roads, and that the abundance of pests would be higher therein, suggesting a cause-effect relationship. The examination of these relationships between roads, birds and pests is needed to create management measures to maintain and improve predator-prey relationships which, in turn, provide ecosystem services.

4. Materials and methods

4.1 Study area

The study was carried out between December 2019 and July 2020, in Companhia das Lezírias S.A, Portugal, a state-owned agrosilvopastoral farm, covering ca. 18,000 ha. Companhia das Lezírias S.A is inserted in the Tagus Estuary Nature Reserve as well as protected by the Special Protection Zone (SPA, Habitats Directive), due to its richness in bird species, and has forest management certified by an international standard (Companhia das Lezírias, n.d.). The sampling sites were all located inside the cork oak area (Figure 4.1). The cork-oak systems occupy 6.603 ha with *Quercus suber* being the dominate specie, known as "montado" in Portugal. The climate in this region is characterized as Mediterranean with hot and dry summers and moderate rainy winters. The annual mean temperature is 16.3ºC, with an annual precipitation of 662.5 mm and summer precipitation of 26.1 mm (Companhia das Lezírias, n.d.). Mediterranean oak woodlands have a rich and diverse community of birds both in spring and winter (Godinho and Rabaça 2011; Leal et al. 2011; Pereira et al., 2015). There are 110 species of birds possible to observe in the study area and six of these species have conservation status (Pereira et al., 2015; Companhia das Lezírias, n.d.). Mediterranean oak woodlands are composed by a mosaic of different forest fields and small areas of shrubs in the understory with non-intensive livestock farming and cork production, making it a suitable and crucial ecosystem to the preservation of breeding and wintering birds (Bugalho et al., 2011; Leal et al. 2011; Pereira et al., 2015; Companhia das Lezírias, n.d.). Furthermore, Companhia das Lezírias S.A is not freely accessible to the general public, and therefore the human perturbation is generally minute. There are reports in the study area of outbreaks of cork oak pest since the beginning of the 20th century, some of the most common case of pests being buprestids *Coroebus florentinus* and *Coroebus undatus* (Coleoptera, Buprestidae), the moths *Tortrix viridana* and *Lymantria dispar* (Lepidoptera) and the chewer sawflies *Periclista Andrei* (Hymenoptera, Tenthredinidae) (Baeta-Neves et al., 1972; Tiberi et al., 2016). However, it is important to point out that *Coroebus undatus* is the one that has the greatest economic effects.

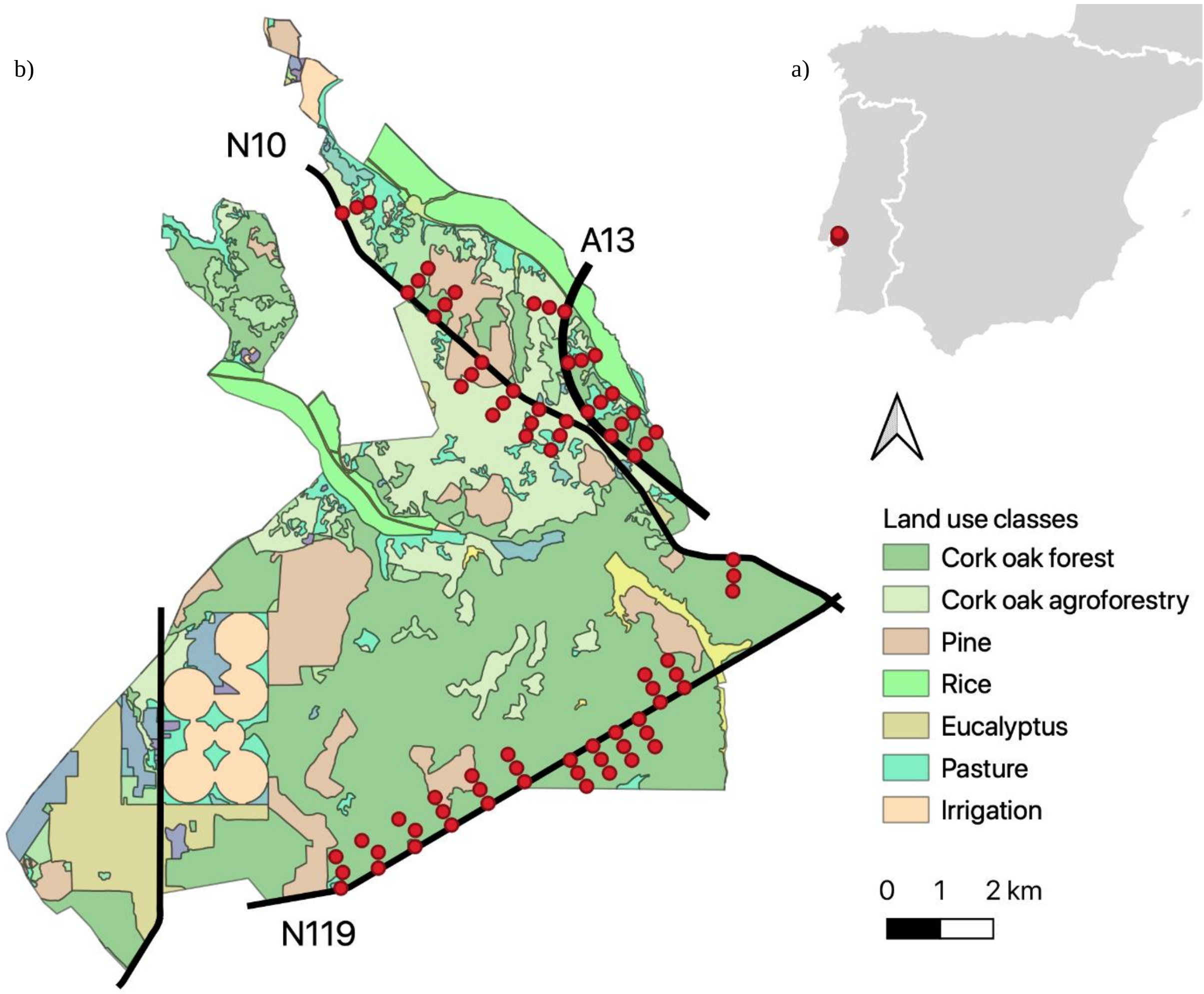

Figure 4.1. Map of the study area – a) Map of Portugal with the red point representing the location of the study area; b) Each red point on the map represents a sampling point. In b) are illustrated the most common land use types in the study area, listed in the figure.

4.2 Sampling sites selection

Sampling sites for bird and pest surveys were selected to be at least 500 m apart to guarantee spatial independence. Sampling sites were established in the proximity of the main paved roads (N119, N10 and A13). Twenty-five sampling sites were selected, each one with three sampling points at increasing distances perpendicular from the road – 30, 300 and 600 meters (total 75 sampling points). All sampling points were surveyed once in winter (December 9, 2019 – January 29, 2020). The objective was to sample all sites again in the following Spring to include wintering birds and breeding season, when males are more active to defend their territory and present a reduced mobility imposed due to breeding constrains. However, logistic constrains due to COVID-19 pandemic (lockdown), forced a reduction in sampling period, and consequently a reduction of the number of sampling site and distance between sampling points. Eleven sites of the twenty-five sites previously sampled, were surveyed during spring (June 3, 2020 – June 20, 2020), at 30 and 600 m from the road (total 22 sampling points in road N10 and N119). Pest surveys were performed in the same sampling points as the spring bird surveys, to assess if road proximity affects the assembly and structure of the predator and prey communities.

4.3 Bird surveys

Ten minutes point-counts were performed to estimate the richness and abundance of birds, recording all birds seen or heard within a 50 m radius. This distance was used to minimize the effects of traffic noise in detectability (Cooke et al., 2020a). Although some perturbation of traffic in point counts performed at 30 m was present, the traffic flow was not continuous allowing the recognition of birds calls. Moreover, survey period of 10 min was used to allow for good recognition of birds at shorter road distance as in point counts performed at higher distances, all species were generally recorded within the first 5 min of survey. Furthermore, previous experiments demonstrated that there is no strong effect of low frequency background noise (traffic) on the observer's ability to detect birds singing in a 50 m radius in the mixed wood forest (Pacifici et al., 2008). Bird counting was conducted by one or two observers within the four hours after sunrise, when birds are more active. Counts started two minutes after arriving to the point to minimize the disturbance made by the observer's arrival and to allow birds to settle down. No bird censuses were carried out under rainy or windy conditions as that could directly affect the bird activity.

4.4 Pest surveys

Pest abundance was measured only in Spring (April 20, 2020 – May 12, 2020), since insect defoliators adapt their life cycle to match the blossoming of leaves and in Portugal oak leaves start to emerge in April (Oliveira et al., 1994; Ivashov et al.2002). In each sampling point, five cork-oaks, with similar age, were randomly selected for pest sampling. Selected trees had no connected canopies to reduce the effect of neighbouring trees on pest occurrence (Ruiz-Carbayo et al., 2017). On each selected tree, 60 leaves (of that year) were collected (state 2 according to Wesołowski and Rowinski, 2006; states D1 - D2 according to Rodríguez Barbero, 2009), half from each north/south side of the canopy, due to possible variation in temperature, moisture, and light. Every set of 30 leaves was considered a sample, stored in a labelled paper bag, and kept in a refrigerator at 5ºC until the moment of analysis to reduce insect activity and preserve the leaves (Pereira et al., 2019). To estimate pest abundance, the leaves were numbered and analysed in search of signs from eleven cork oak pests, based on Pereira et al (2015) (Figure 4.2 and Figure 4.3), oak leaf-roller weevil *Attelabus nitens* (Coleoptera, Curculionidae), cochonilha *Asterodiaspis ilicicola* (Hemiptera, Asterolecaniidae), gall-maker *Dryomia lichtensteinii* (Diptera, Cecidomyiidae), leaf cutters moths (Lepidoptera), chewer sawflies *Periclista andrei* (Hymenoptera, Tenthredinidae), weevil-miners sp I (Coleoptera, Curculionidae), weevil-miners sp II (Coleoptera, Curculionidae), miners caterpillars Nepticulidae (Lepidoptera, Nepticulidae), midrib-miners (Lepidoptera, Heliozelidae), miners caterpillars Gracillaridae (Lepidoptera, Gracillaridae) and coleoptera miners (Coleoptera, Curculionidae), and recorded the number of leaves with sign of herbivory for each group of pests (Pereira et al., 2019). Leaf assessment was carried out up to one day after the leaves were collected and in the same order in which they were collected. So that during leaf assessment the leaves are in conditions similar to the collection.

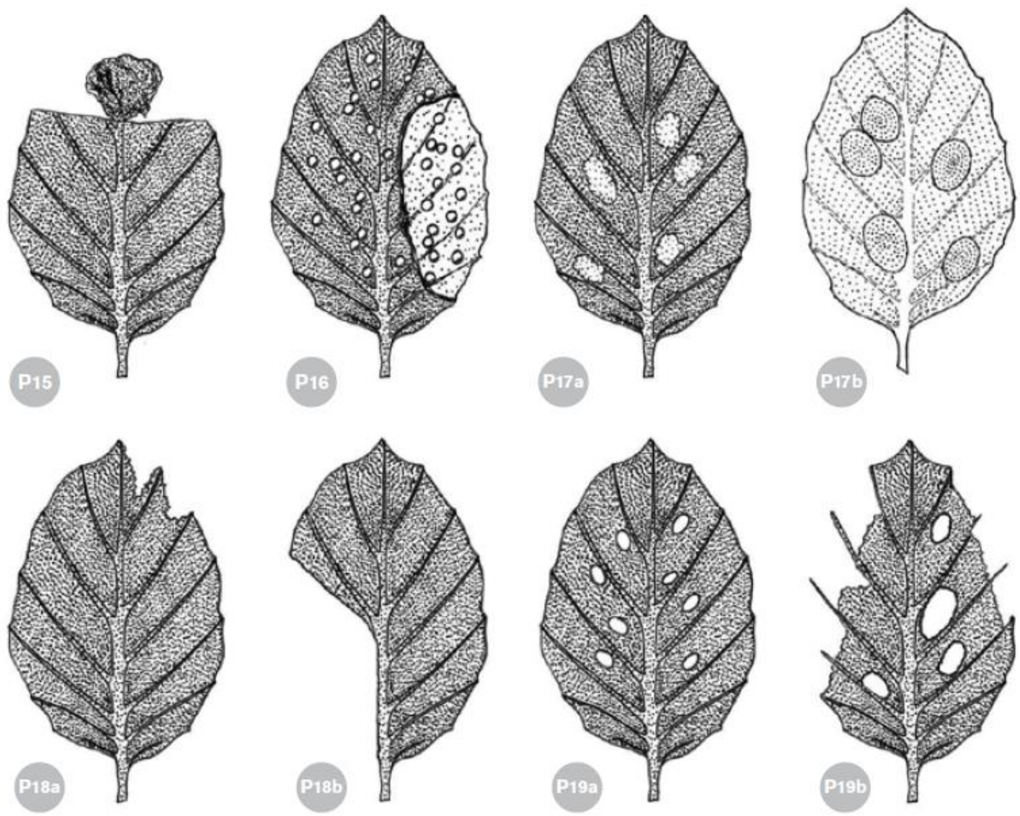

Figure 4.2. Signs of cork oak pest: oak leaf-roller weevil (P15), cochonilha (P16), gall-maker (Top of leaf P17a, bottom of leaf P17b), leaf cutters moths (P18), chewer sawflies (P19) (Pereira et al., 2015). Permission was requested to the author to use this image.

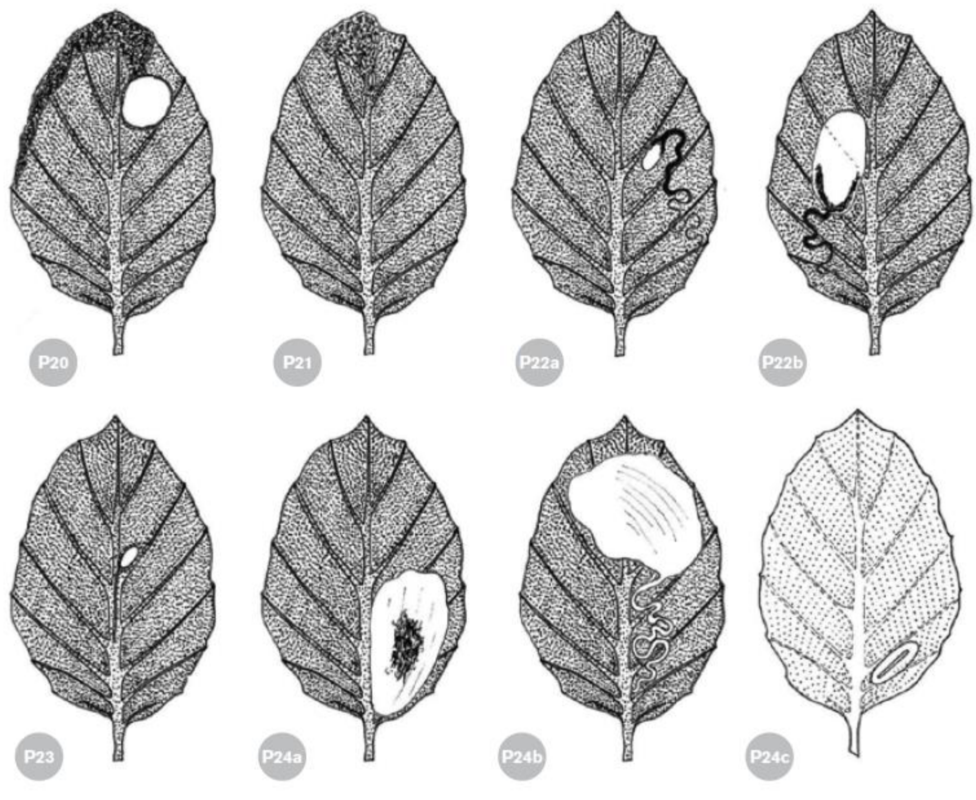

Figure 4.3. Signs of cork oak pest: weevil-miners sp I (P20), weevil-miners sp II (P21), miners caterpillars Nepticulidae (P22), midrib-miners (P23), miners caterpillars Gracillaridae (P24) (Pereira et al., 2015). Permission was requested to the author to use this image.

In the same sampling points, we collected data regarding two species of buprestids, *Coroebus florentinus* and *Coroebus undatus*. The presence of *Coroebus florentinus* (Coleoptera, Buprestidae) was assessed through the identification of the number of dead branches due to larvae activity in the tree (Cárdenas and Gallardo, 2012) (Figure 4.4). Characterization was performed according to criteria described by Soria Iglesias (1990), the presence of more than four branches per tree suggest a greater state of degradation. Accordingly, characterization was performed in four ordered classes regarding the degree of infection by this pest: absent, when there were no infected branches; low, when the tree had up to two infected branches; medium, when it had three or four infected branches; high, when it had more than four branches with signs of *C. florentinus*. Finally, it was also recorded whether the tree trunk showed signs of *Coroebus undatus* (Coleoptera, Buprestidae) by observing if there were galleries in the trunk (Figure 4.5) made by larval in the north and south sides of tree. The trunk was not evaluated as a whole, because the degrees of this species are considered high when it is possible to observe galleries in both sides including the side with less exposure to the sun (Soria Iglesias 1990). Thus, the signs of *C. undatus* were assessed according to criteria described by Pereira et al. (2015), using three ordered categories: trunk not affected; one side affected; both sides affected.

Figure 4.4. Signs of *Coroebus florentinus* in the canopy of a cork oak tree.

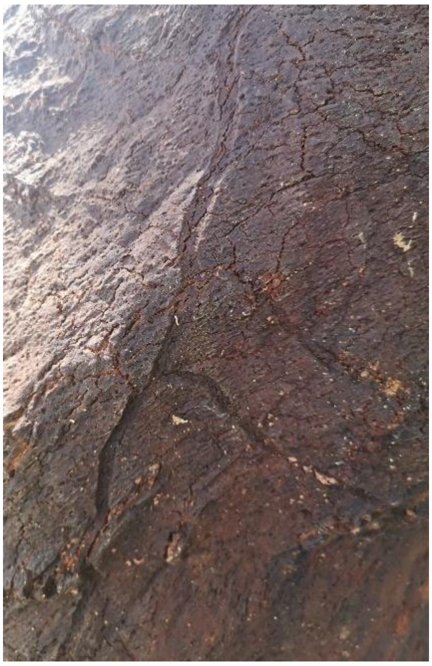

Figure 4.5. Signs of *Coroebus undatus* in the trunk of a cork oak tree.

4.5 Data analysis

4.5.1 Bird occurrence patterns

To model bird occurrence patterns, only species known to feed on invertebrates were considered, discarding all species that have no invertebrates in their diet, filtered according to the Elton traits database (Wilman et al., 2014). This resulted in 41 species retained (See Table 1, Appendix) and hereafter will only refer to those. Due to the impossibility of distinguish between the vocalizations of the two starling species (Sturnus unicolor and Sturnus vulgaris), both occurring in the study area, they were thus classified as Sturnus sp. We modeled three types of bird occurrence patterns across sampling points, namely bird abundance, the total number of birds observed per sampling point in each season; bird richness, the total number of species detected per sampling point in each season; and species presence-absence, a specie was considered as present in each sampling point if occurred at least once.

The results were considered significant when p-value < 0.05. Statistics were done using R version 3.6.3 (R Core Team, 2020). Negative binomial regression models were built using the R package 'MASS' (Venables and Ripley, 2002), relating bird abundance / richness with distance to road (30 m, 300 m and 600 m), road identification (N10, N119 or A13) and season (spring or winter). Model performance was assessed through Nagelkerke R^2 using the R package 'rsq' (Zhang, 2020) and the model with the highest value of Nagelkerke R^2 was selected. Generalized Linear Models (binomial) were built for each bird species presence-absence using the same set of predictors. It was built separated models for each season (winter and spring) due to the difference in number of sampling points performed between them. The results were considered significant when p-value < 0.05.

4.5.2 Pest occurrence patterns

Pest abundance (log-transformed) was related to the distance to the road (30 m and 600 m), road identification (N10 and N119) and side of the canopy (north side or south side) using a linear mixed effect model. Tree identification nested in sampling point identification was included as a random factor. Generalized linear mixed-effects models (negative binomial) were built for each group of cork oak pests using the same set of predictors, including tree identification nested in sampling point identification as a random factor. The occurrence of *C. florentinus* and *C. undatus* was related to the distance to the road using an ordinal logistic regression. For the occurrence of these two species of buprestids a different statistical method was used, since unlike the data for each group of cork oak pests (counts), the response variable is an ordinal variable. The results were considered significant when p-value < 0,05. Statistics were done using R version 3.6.3 (R Core Team, 2020). Linear mixed effect model and Generalized linear mixed-effects models were performed using the 'lme4' (Bates et al., 2015) and 'lmerTest' R packages (Kuznetsova et al., 2017). Ordinal logistic regression was performed using the 'polr' command from the R package 'MASS' (Venables and Ripley, 2002) and the R package 'effects' to create effect displays for the models (Fox and Weisberg, 2019).

5. Results

5.1 Bird occurrence patterns

A total of 1673 individuals from 35 bird species were observed in winter and 337 individuals from 37 bird species were recorded in spring. The most abundant bird species in winter were Common chaffinch *Fringilla coelebs*, Common Chiffchaff *Phylloscopus collybita* and Blue Tit *Cyanistes caeruleus* and in the spring were Blue Tit, Common Chaffinch and European goldfinch *Carduelis carduelis*. In general, bird abundance (considering all species) was higher as the distance to the road increased in both seasons and in the different roads (Figure 5.1). The model, regarding bird abundance showed a significant positive association with distance to the road (PC 300m and PC 600m), and a significant lower abundance in N10 relatively to A13. There were no significant differences between A13 and N119. There were also no differences between seasons (winter and spring) and with observers. The R^2 for the bird abundance model was 0.539. A summary of the associations between bird abundance and distance to road, road identification or season is present in Table 5.1.

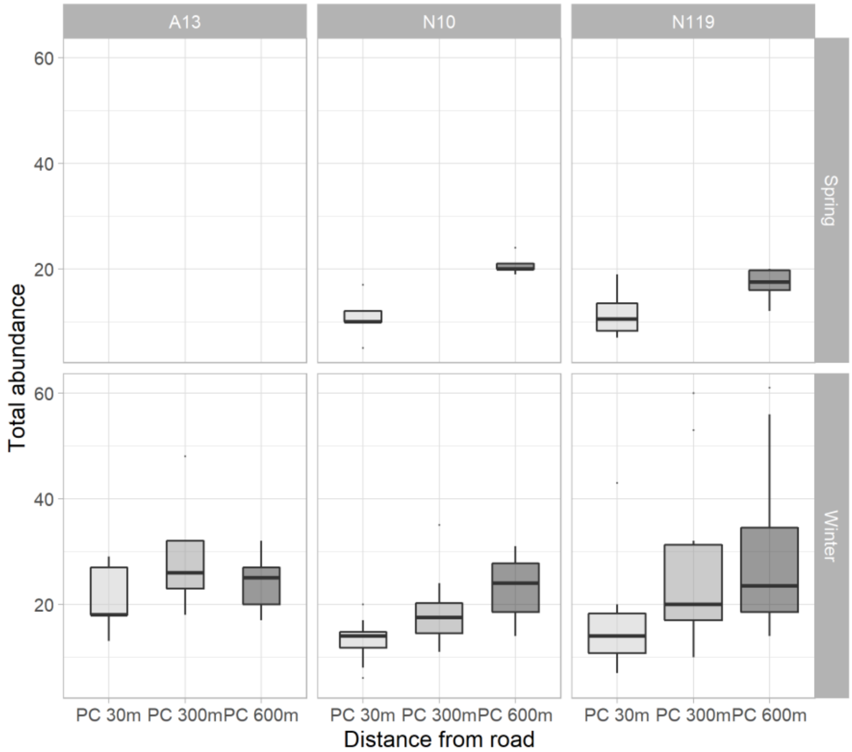

Figure 5.1. Bird abundance at increasing distance to the road, per surveyed road and season. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600 m, respectively.

Table 5.1. Summary of regression model (negative binomial) relating bird abundance with distance to the road, road identification (A13, N10, N119), season and observers. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600 m, respectively.

	Bird abundance				
Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	2.53	0.16	15.97	2.22 – 2.84	**<0.001**
Observers	0.17	0.10	1.65	-0.03 – 0.38	0.098
PC 30m	*Reference*				
PC 300m	0.42	0.11	3.92	0.21 – 0.64	**<0.001**
PC 600m	0.51	0.10	5.31	0.32 – 0.69	**<0.001**
A13	*Reference*				
N10	-0.36	0.14	-2.63	-0.63 – -0.09	**0.008**
N119	-0.21	0.13	-1.55	-0.47 – 0.05	0.122
SeasonWinter	0.20	0.12	1.59	-0.05 – 0.44	0.113
Observations	97				
R^2 Nagelkerke	0.539				

In general, bird richness (considering all species) was higher as the distance to the road increased in both seasons and in the different roads, except for road A13 (Figure 5.2). The model, regarding bird richness revealed a significant positive association with distance to the road relatively to 30 meters, but only at 600 m from the road (PC 600 m). There was not a significant difference in bird richness between 30 m (PC 30m) and 300 m (PC 300 m). Bird richness also revealed a significant positive association with observers. There was a significant lower richness in N10 relatively to A13. There were no significant differences between N119 and A13. The R^2 for the bird richness model was 0.375. A summary of the associations between bird richness and distance to road, road identification or season is present in Table 5.2.

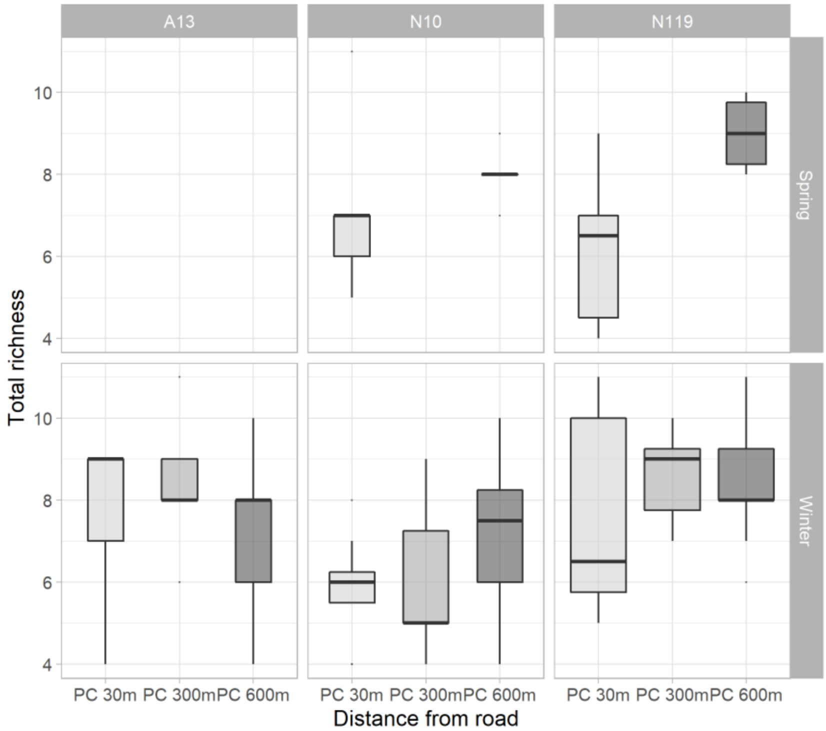

Figure 5.2. Bird richness at increasing distance to the road, per surveyed road and season. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600 m, respectively.

Table 5.2. Summary of regression model (negative binomial) relating bird richness with distance to the road, road identification (A13, N10, N119), season and observers. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600m, respectively.

	Bird richness				
Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	1.88	0.14	13.16	1.60 – 2.16	**<0.001**
Observers	0.26	0.10	2.62	0.07 – 0.46	**0.009**
PC 30m	*Reference*				
PC 300m	0.13	0.10	1.34	-0.06 – 0.33	0.181
PC 600m	0.17	0.09	1.98	0.00 – 0.34	**0.047**
A13	*Reference*				
N10	-0.29	0.13	-2.23	-0.55 – -0.03	**0.026**
N119	-0.14	0.13	-1.11	-0.39 – 0.11	0.268
SeasonWinter	-0.20	0.11	-1.77	-0.43 – 0.02	0.076
Observations	97				
R^2 Nagelkerke	0.375				

The models, regarding the presence-absence of 41 species across sampling points, revealed significant association with distance to the road in three species. Two species, Sardinian warbler *Sylvia melanocephala* (PC 600m); European nuthatch (PC 300m and PC 600m) had a higher presence as the distance to the road increased during winter (Table 2, Appendix). Only one specie, European stonechat *Saxicola rubicola*, showed significant higher presence in proximity to roads in both seasons (Table 2, Appendix). However, three other bird species had a low p-value close to 0.05 regarding distance to the road (Table 3, Appendix). European goldfinch (p-value= 0.07) and Woodlark *Lullula arborea* (p-value=0.06) had a higher presence as the distance to the road increased during winter. Corn bunting *Emberiza calandra* (p-value=0.09) showed higher presence close to roads during winter.

All models for species presence-absence that showed significant associations with road identification and observers can be found in the Table 4, Appendix.

5.2 Pest occurrence patterns

A total of 2640 cork oak leaves were analysed. The most common orders found were Lepidoptera and Coleoptera. It was possible to identify signs of eight groups of pests affecting oak leaves: oak leaf-roller weevil *Attelabus nitens* (Coleoptera, Curculionidae), gall-maker *Dryomia lichtensteinii* (Diptera, Cecidomyiidae), leaf cutters moths (Lepidoptera), chewer sawflies *Periclista andrei* (Hymenoptera, Tenthredinidae), weevil-miners (Coleoptera, Curculionidae, two species), midrib-miners (Lepidoptera, Heliozelidae) and coleoptera miners (Coleoptera, Curculionidae). Signs of leaf cutters moths and chewer sawflies were the most found with a percentage of 62.5% and 52.5% affected leaves, respectively (Table 5.3).

Table 5.3. Number and percentage of affected leaves for each group of cork oak pests registered during sampling.

Cork oak pest	Number of affected leaves	Percentage of affected leaves (%)
Oak leaf-roller weevil	16	0.6
Gall-maker	73	2.8
Leaf cutters moths	1651	62.5
Chewer sawflies	1385	52.5
Weevil-miners sp. I	32	1.2
Weevil-miners sp. II	36	1.4
midrib-miners	204	7.7
Coleoptera miners	106	4.0

In general, pest abundance (considering all species) was lower as the distance to the road increased, in both roads (Figure 5.3). The model, regarding pest abundance revealed a significant negative association with distance to the road (PC 600m). There were no significant differences in pest abundance between side of the canopy or roads (Table 5.4).

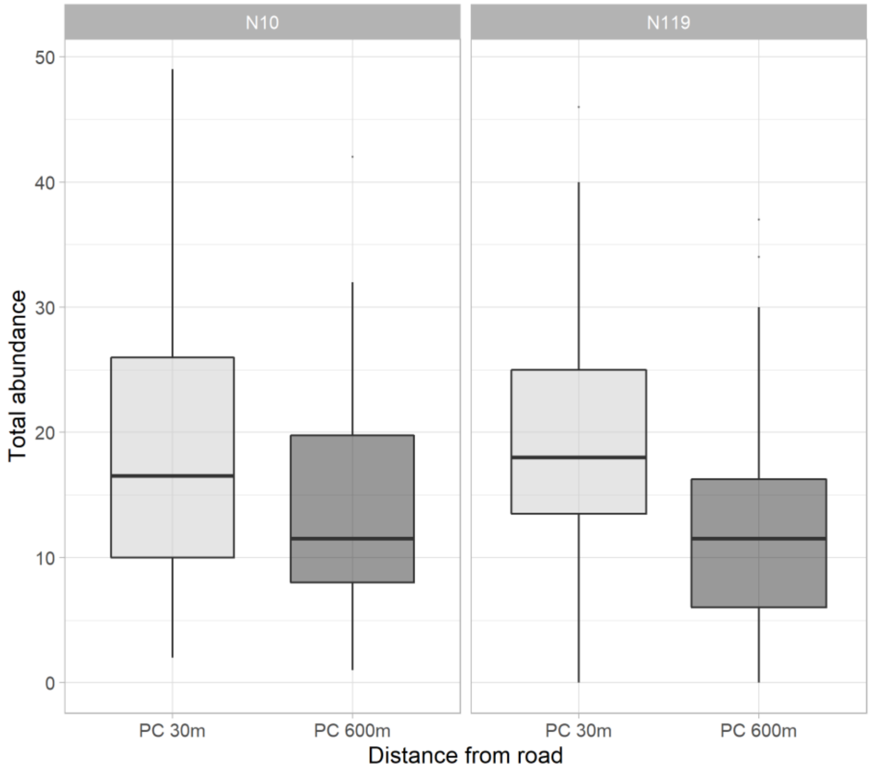

Figure 5.3. Pest abundance at increasing distance to the road, per surveyed road. PC 30m and PC 600m stand for sampling points at 30 and 600 m, respectively.

Table 5.4. Summary of linear mixed effect model relating pest abundance with distance to the road, road identification (N10 and N119) and side of the canopy (north side and south side). PC 30m and PC 600m stand for sampling points at 30 and 600 m, respectively.

	Pest abundance				
Predictors	*Estimates*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	2.87	0.16	17.85	2.55 – 3.18	**<0.001**
PC 30m	*Reference*				
PC 600m	-0.44	0.11	-4.05	-0.66 – -0.23	**<0.001**
North	*Reference*				
South	0.02	0.07	0.28	-0.12 – 0.16	0.781
N10	*Reference*				
N119	-0.09	0.20	-0.44	-0.48 – 0.30	0.659
Random Effects					
σ^2	0.30				
$\tau_{00\ TreeID:Site}$	0.18				
$\tau_{00\ Site}$	0.07				
ICC	0.46				
N_{TreeID}	110				
N_{Site}	11				
Observations	220				
Marginal R^2 / Conditional R^2	0.085 / 0.510				

The models relating the abundance of each group of cork oak pest across sampling points (Table 5.5), revealed a higher abundance of leaf cutters moths, chewer sawflies and gall-maker in trees close to the road. Regarding side of canopy, oak leaf-roller weevil and weevil-miners sp.II revealed a higher abundance in the south of the canopy and gall-maker in the north side. Gall-maker was the only pest to show an association between abundance and the roads surveyed. Gall-maker revealed a lower abundance in N119, relatively to N10. The abundance of weevil-miners sp.I, midrid-miners and Coleoptera miners was not affected by neither of the variables analysed.

Table 5.5. Summary of generalized linear mixed-effects models relating each cork oak pest group with distance to the road and side of the canopy (north side and south side). PC 30m and PC 600m stand for sampling points at 30 and 600 m,

Leaf cutters moths

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	2.17	0.13	16.82	1.92 – 2.42	**<0.001**
PC 30m	*Reference*				
PC 600m	-0.45	0.12	-3.69	-0.68 – -0.21	**<0.001**
N10	*Reference*				
N119	-0.15	0.15	-1.01	-0.43 – 0.14	0.310
North	*Reference*				
South	-0.01	0.08	-0.07	-0.17 – 0.16	0.948
Random Effects					
σ^2	0.32				
τ_{00}	$0.20_{\text{TreeID:Site}}$				
	0.02_{Site}				
ICC	0.41				
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.093 / 0.461				

Chewer sawflies

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	1.86	0.18	10.22	1.50 – 2.22	**<0.001**
PC 30m	*Reference*				
PC 600m	-0.43	0.12	-3.51	-0.67 – -0.19	**<0.001**
N10	*Reference*				
N119	0.00	0.22	0.02	-0.42 – 0.43	0.984
North	*Reference*				
South	0.11	0.11	0.99	-0.10 – 0.31	0.322
Random Effects					
σ^2	0.48				
τ_{00}	$0.10_{\text{TreeID:Site}}$				
	0.09_{Site}				
ICC	0.28				
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.070 / 0.331				

Oak leaf-roller weevil

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	-10.80	2.57	-4.21	-15.83 – -5.77	**<0.001**
PC 30m	*Reference*				
PC 600m	-0.28	2.34	-0.12	-4.86 – 4.30	0.904
N10	*Reference*				
N119	-0.65	2.36	-0.27	-5.27 – 3.98	0.784
North	*Reference*				
South	2.70	1.03	2.63	0.69 – 4.72	**0.009**
Random Effects					
σ^2	9.21				
τ_{00}	$64.00_{\text{TreeID:Site}}$				
	0.00_{Site}				
ICC					
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.176 / NA				

Gall-maker

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	-5.42	0.01	-993.76	-5.43 – -5.41	**<0.001**
PC 30m	*Reference*				
PC 600m	-1.01	0.01	-185.08	-1.02 – -1.00	**<0.001**
N10	*Reference*				
N119	-0.23	0.01	-41.41	-0.24 – -0.22	**<0.001**
North	*Reference*				
South	-0.87	0.01	-159.51	-0.88 – -0.86	**<0.001**
Random Effects					
σ^2	6.52				
τ_{00}	$26.81_{\text{TreeID:Site}}$				
	0.00_{Site}				
ICC	0.80				
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.014 / 0.807				

Weevil-miners sp.I

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	-3.89	1.00	-3.88	-5.86 – -1.92	**<0.001**
PC 30m	*Reference*				
PC 600m	-1.07	0.65	-1.63	-2.35 – 0.21	0.102
N10	*Reference*				
N119	0.88	0.69	1.27	-0.47 – 2.23	0.202
North	*Reference*				
South	0.51	0.37	1.38	-0.21 – 1.23	0.166
Random Effects					
σ^2	4.20				
τ_{00}	$3.81_{\text{TreeID:Site}}$				
	0.09_{Site}				
ICC	0.48				
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.063 / 0.514				

Weevil-miners sp.II

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	-4.57	1.02	-4.46	-6.58 – -2.56	**<0.001**
PC 30m	*Reference*				
PC 600m	-0.01	0.65	-0.02	-1.28 – 1.26	0.983
N10	*Reference*				
N119	0.48	0.72	0.66	-0.93 – 1.89	0.507
North	*Reference*				
South	0.94	0.38	2.51	0.21 – 1.68	**0.012**
Random Effects					
σ^2	3.88				
τ_{00}	$4.52_{\text{TreeID:Site}}$				
	0.22_{Site}				
ICC	0.55				
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.031 / 0.564				

Midrib-miners

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	-0.21	0.26	-0.83	-0.72 – 0.29	0.408
PC 30m	*Reference*				
PC 600m	-0.17	0.26	-0.67	-0.68 – 0.33	0.503
N10	*Reference*				
N119	-0.14	0.26	-0.54	-0.65 – 0.37	0.588
North	*Reference*				
South	-0.12	0.21	-0.55	-0.53 – 0.30	0.585
Random Effects					
σ^2	1.28				
τ_{00}	$0.52_{\text{TreeID:Site}}$				
	0.00_{Site}				
ICC					
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.012 / NA				

Coleopetra miners

Predictors	*Estimate*	*std. Error*	*Statistic*	*CI*	*p-value*
(Intercept)	-0.27	0.37	-0.73	-0.99 – 0.45	0.467
PC 30m	*Reference*				
PC 600m	-0.08	0.37	-0.21	-0.80 – 0.64	0.833
N10	*Reference*				
N119	-0.48	0.37	-1.31	-1.19 – 0.24	0.191
North	*Reference*				
South	-0.43	0.36	-1.18	-1.14 – 0.28	0.238
Random Effects					
σ^2	2.07				
τ_{00}	$0.00_{\text{TreeID:Site}}$				
	0.00_{Site}				
ICC					
N	110_{TreeID}				
	11_{Site}				
Observations	220				
Marginal R^2 / Conditional R^2	0.048 / NA				

Regarding *C. florentinus*, the probability of trees showing no infection, or a low degree of infection increased with distance. The probability of trees having medium or high degrees of infection decreased with distance. Also, the probability of trees showing high degrees of infection at 600 m from the road was lower than the probability for the other degrees of infection (Figure 5.4). These patterns occurred in both roads surveyed (N10 and N119) (Figure 5.5). However, in road N10 the probability of trees presenting medium or high degrees of infection was higher than in road N119. Also, it was less probable for trees to present low degrees of infection or no infection in road N10.

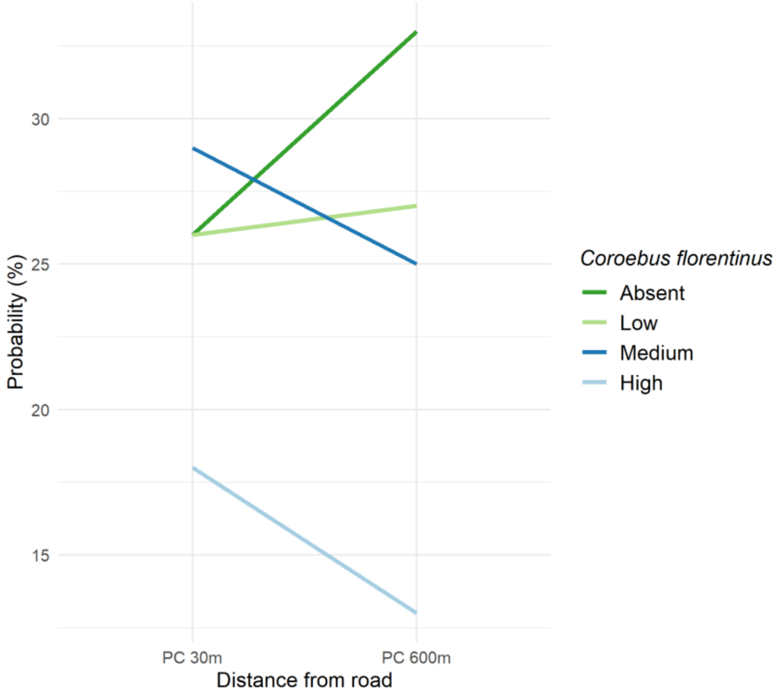

Figure 5.4. Probabilities of infection by *Coroebus florentinus* at increasing distance to the road. PC 30m and PC 600m stand for sampling points at 30 and 600 m, respectively. Classes of degree of infection were Absent= no infected branches; Low= up to two infected branches; Medium= three or four infected branches; High=more than four infected branches.

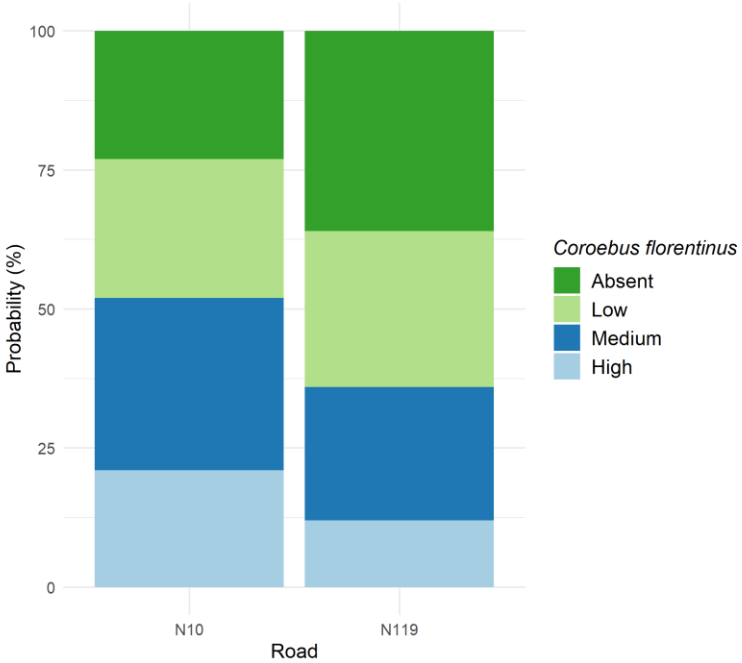

Figure 5.5. Probabilities of infection by *Coroebus florentinus* at the two roads surveyed (N10 and N119). Classes of degree of infection were Absent= no infected branches; Low= up to two infected branches; Medium= three or four infected branches; High=more than four infected branches.

The probability of trees not being infected with *C. undatus* increased with the distance and the probability of trees presenting signs of infection, either in one side or in both sides of the trunk, decreased with distance. However, the decreased in the probability of infection in one side of the trunk as distance increased was not so severe. The probability of trees having both sides affected by *C. undatus* at 600 m from the road was lower than the probability of trees having one side affected (Figure 5.6). These patterns can be seen in both roads surveyed (N10 and N119) (Figure 5.7). However, in road N10 the probability of trees not being infected was lower than in road N119. Also, it was more probable for trees to show signs of infection in road N10.

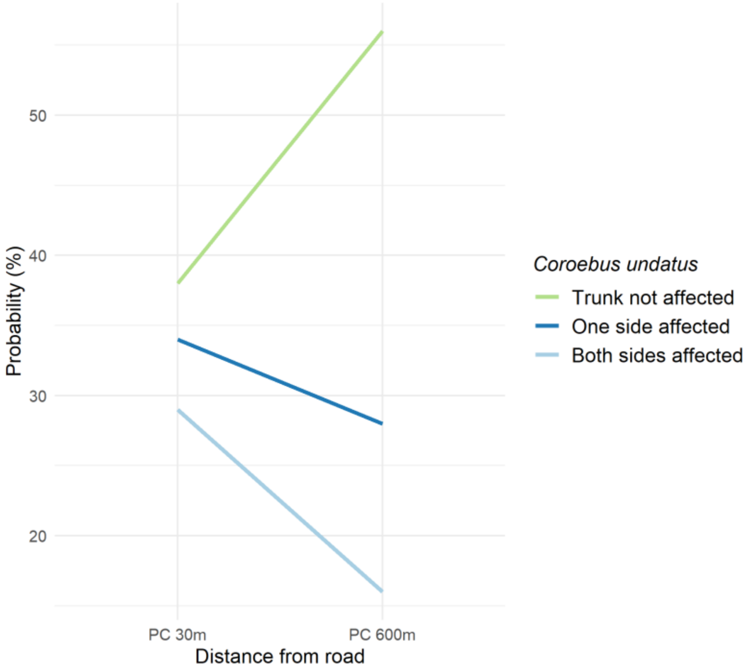

Figure 5.6. Probabilities of infection by *Coroebus undatus* at increasing distance to the road. PC 30m and PC 600m stand for sampling points at 30 and 600 m, respectively.

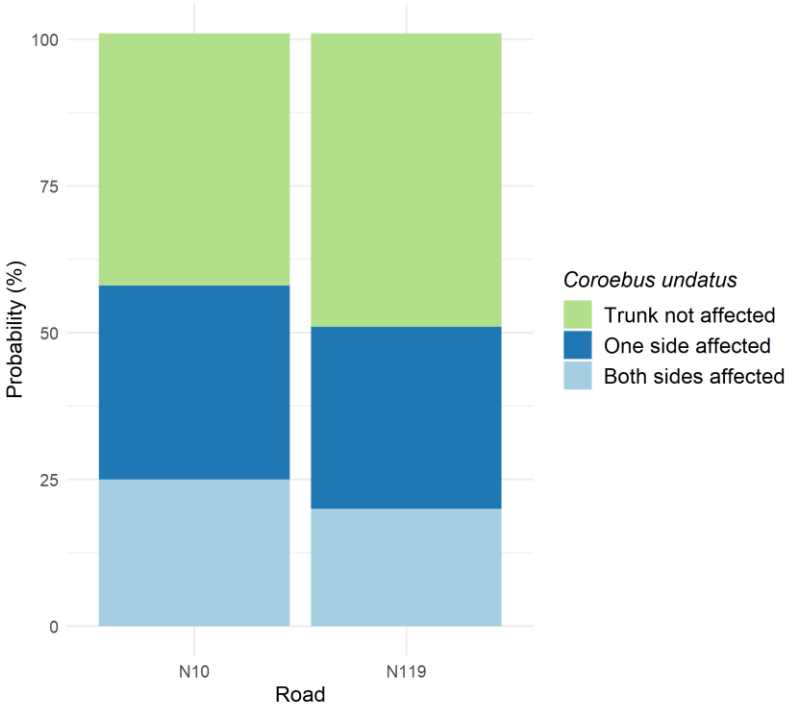

Figure 5.7. Probabilities of infection by *Coroebus undatus* at the two roads surveyed (N10 and N119).

5.3 Bird and pest occurrence patterns

Overall, the results show that close to the road (PC 30m) pest abundance was higher, whereas bird abundance was lower and farther away from the road (PC 600m) bird abundance was higher, whereas pest abundance was lower, in both roads (Figure 5.8).

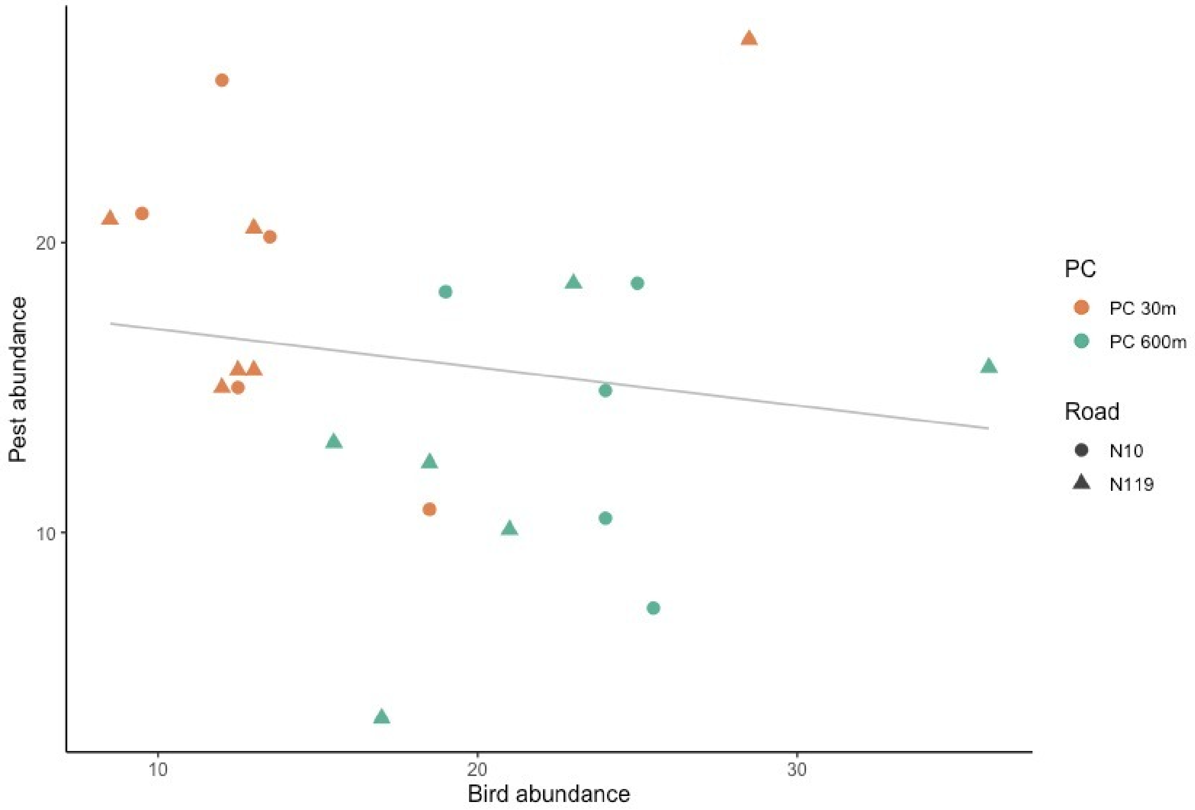

Figure 5.8. Relation between pest abundance and bird abundance at increasing distance to the road, per surveyed road. PC 30m and PC 600m stand for sampling points at 30 and 600 m, respectively.

6. Discussion

The results indicate that bird abundance and richness decreased with proximity to roads, with four bird species showing lower presence near the roads, whereas two species displayed lower presence as the distance to the road increased. The opposite pattern was found for pests, pest abundance increased with proximity to the road, with three species of cork oak pest showing a higher abundance in trees close to roads. Both *C. florentinus* and *C. undatus* showed a decrease in the degrees of infection as the distance to the road increase. Overall, the results show that bird occurrence patterns decrease with road proximity, whereas the abundance of cork oak pest increase, suggesting a cause-effect relation between the two groups, mediated by road effects. In road proximity, lower predation pressure by birds is likely to result in a higher pest abundance in these areas. In this study, the habitat is very similar in all sampling points regardless of the distance to the road, so it is possible to presume that these results found in bird abundance, bird richness and species presence are related to the road itself. These results provide the first evidence that roads may affect the provision of ecosystem services, particularly by disrupting the pest control by birds in cork-oak systems. We anticipate that such impact may translate into important losses to cork economy.

The results are consistent with previous studies as the bird abundance (Rheindt, 2003; Benítez-López et al., 2010; Summers et al., 2011; Cooke et al., 2020b) and richness (Rheindt, 2003; Summers et al., 2011) increased with distance to the road, due to bird populations being more affected at short distances from road (Rheindt, 2003). Usually, highways are the road infrastructures that affect most the abundance and richness of birds (Kuitunen et al., 1998), due to its high degree of traffic. Observations carried out during this study suggest that A13 has little traffic in comparison with other highways. Probably for that reason, the abundance and richness of birds was lowest in sampling points at road N10 rather than A13. Regarding bird richness, it was possible to detect a higher diversity at 600 meters from the road. This variation may occur due to traffic noise reaching its minimum at approximately 450 meters from the road (Summers et al., 2011), allowing bird species that are more susceptible to the road effects to occur.

Four bird species showed a positive association with distance to the road, European nuthatch, Sardinian warbler, European goldfinch and Woodlark. Like the present sudy, Rheindt (2003) found European nuthatch to have lower presence close to roads. Regarding Sardinian warbler studies only showed a higher rate of roadkill than expected from their abundance (Garriga et al., 2012; Santos et al., 2016), from what we can find there were no studies showing Sardinian warbler to have a lower presence close to the road. However, lower bird presence close to roads may be associated with higher roadkill rates, therefore depleting the nearby population, and/or birds avoiding the proximity of roadside habitats. Moreover, roadside areas may have a lower breeding success due to the negative impacts caused by the road, a higher predation risk or a higher perturbation (traffic noise), or all these together, which may restrain birds to occupy those areas. Such reduction in bird occurrence is probably causing a higher pest abundance in road adjoining areas. Unlike the present study, Morelli et al. (2015) found Woodlark and European goldfinch to have a higher presence close to roads. However, Woodlark is positively affected by roads when in open environments, as agricultural areas and grasslands (Morelli et al., 2015) and/or in proximity to road with low traffic and therefore reduce traffic noise (Peris and Pescador, 2004), and these features differ from the are in the present study. According to Morelli et al. (2015), European goldfinch is positively affected by roads when in closed environments, mainly forest and mixed environments, such as the present study area, yet Morelli et al. (2015) collect bird data only during spring. Since the bird data from spring was reduced there is no way to be certain if that affect the result regarding this specie. European stonechat did not show a decline in presence close to the road, instead presence was higher as distance to the road decrease. This outcome was expected since this specie does not show the negative effects of roads (Morelli et al., 2015) as the road provides marginal habitats, hedgerows and vegetation suitable for this specie. Corn bunting showed lower presence as the distance to the road increased. According to Peris and Pescador (2004), this specie tends to be more abundant near roads with high traffic noise. So, although, all surveys were conducted when traffic noise was lower, and observations carried out during this study suggest that traffic noise was not particularly high in the study area. This shows that Corn bunting is adapted to road edge habitat, which may be the reason why this specie showed higher presence close to the road.

Half of the species that revealed associations showed either lower or higher presence as the distance to the road increase. As for the species that did not show a significant association with the distance to the road, some may reflect reality, however some may be due to insufficient data. Although some birds adapt to roadside habitat and even manage to take advantage of these infrastructures, we believe the present results regarding presence of species and distance to the road are underestimated. This occurs most likely because of the reduction in the number of sampling points during spring.

Detectability is an important aspect to consider when planning a methodology for bird surveys since it is known that not all birds around the point-count or transect will be recorded. Especially, close to roads where traffic noise can interfere with the observers' ability to detect the birds calls and lead to errors in estimating bird occurrence patterns and the impact of roads on bird communities (Cooke et al., 2020a). In this work, only early morning surveys were preformed, when traffic was low and not continuous, and 10 minutes surveys were performed to give enough time to detect birds regardless of traffic noise. Also, only birds seen or heard within a 50 meters radius of the sampling point were counted, since there is no strong effect of low frequency background noise (traffic) on the observers' ability to detect birds at that distance (Pacifici et al., 2008). Another aspect to be considered is the observer effect, since the level of experience between observers can cause differences in the number of individuals and species detected (Eglington et al., 2010). Therefore, it is important to incorporate observers as a variable in bird analyses, to account for a possible bias due to observer effect (Eglington et al., 2010). The present results showed a positive association between bird richness and observers, but there was no association between bird abundance and observers. So, it is possible that there was some observer effect in bird richness analyses.

Pest abundance decreased with distance to the road, as bird abundance increased. The present results indicate that this decreased in pest abundance may occur due to a stronger predation pressure by birds that result in lower abundance of pests in oak canopies, as similar results regarding predation pressure were found for chewer caterpillar in Ceia et al. (2016) and for insect herbivores in Bereczki et al. (2014).

The probability of trees showing no infection or a low degree of infection by *C. florentinus* and *C. undatus* increased with distance, and the opposite for medium or high infections. These results suggest that the buprestids abundance may be related to a lower abundance of predators closer to the road, since the medium and high degrees of infection decreased with distance to the road, as bird abundance increased, and the degree of infection was higher in road N10 where bird abundance was lower. *C. undatus* is one of the most worrying pests in Mediterranean oak woodlands since this specie attacks only cork oaks and can jeopardize the stripping (Pereira et al., 2015). The degree of infection for this species is considered high when it is possible to observe galleries in both sides of the tree and the present results show the probability of trees having both sides affected by *C. undatus* farther away from the road was particularly low. Also, according to Soria Iglesias (1990) one side of the tree affected is the maximum tolerable for the sustainability of the cork oak population. Which is collaborated with the present results since the decreased in the probability of infection in one side of the trunk as distance increased was not so severe. Regarding *C. florentinus*, the tree is considered to have a greater state of degradation when it has more than four branches affected (Soria Iglesias, 1990). In the present study, the probability of trees presenting high degree of infection (more than four branches) by *C. florentinus* farther away from the road was lower than the other degrees. Since bird abundance was higher farther away from the road, this evidence related to both species of buprestids can demonstrate the importance of birds to control pest population.

Until now, no study assessed the abundance of bird and pest considering the distance to road. This approach allowed us to identify a possible a cause-effect relation between the two groups mediated by road effects. Therefore, it is important to conduct further studies to better understand how road proximity affects the assembly and structure of the predator and prey communities in Mediterranean oak woodlands and the variation in patterns, visible in one sampling point of this study.

Our planet consists of a vast network of ecosystems and can be difficult to understand or evaluate to which extent we are dependent on the proper functioning of these ecosystems and the vital services they provide to humanity. The present study pointed out how important relationships are formed among the elements of the Mediterranean oak woodlands (roads, birds, and cork oak pests), which are linked by predator-prey relationships. Studies as this are important as they help to rethink land use when planning the construction of new roads and the possibility of creating new mitigation plan for existent roads. In the present study, roads directly influenced the abundance of birds, and thereby had an indirect effect on oak pest abundance. One major problem in controlling insect pest in Mediterranean oak woodlands is the difficulty in predicting their outbreaks. Since birds are an effective way of controlling pest population and prevent possible outbreaks, it is important to establish measures and/or recommendations to mitigate the negative effects of roads in bird communities. Consequently, by maintaining a stable population of specialist and generalist predators, counteract the current scenario of declining in cork oaks due to pests and protect the biodiversity of this ecosystem. Some mitigation measures to improve bird populations close to roads are installation of flight diverters to reduce mortality due to bird-vehicle collisions (Kociolek et al., 2015), by preventing a direct access to the road and forcing birds to fly higher. This flight diverters can be poles to produce a physical barrier, flags, fences, wide posts and soil berms higher than the road (Kociolek et al., 2015). Avoid scheduling the constructions and maintenance of roads, during critical period for bird, such as migration and breeding, as birds are more vulnerable, and these activities can compromise their well-being (Kociolek et al., 2015). Introduction of crossing structures, either underpasses or overpasses, to improve traffic safety and increase habitat connectivity (Kociolek et al., 2015; Rytwinski and Fahrig, 2015). Furthermore, the introduction of flight diverters and crossing structures can complement each other, since hedges can direct animal toward crossing structures. Creation of a quieter types of pavements and tires to reduce effects of traffic noise (Rytwinski and Fahrig, 2015). Development of new nesting and shelter sites, by adapting existing artificial structures or introduction of new structures, such as nest boxes (Pereira et al., 2015). The installation of artificial nest boxes is an effective and simple measure to improve bird population, especially the breeding populations of hole nesting birds. Nest boxes function as artificial cavities for a wide range of birds, allowing for a mitigation of population declines and, in long-term, increasing the bird population (Pereira et al., 2015). These nest boxes can be made of virgin cork and pieces of low commercial value cork, allowing a better microclimate amenity inside (Pereira et al., 2015). Furthermore, studies of Great tit populations showed that after the installation of nest boxes these birds were able to respond to outbreaks of Pine processionary moth (Pimentel and Nilsson, 2007) and reduce damage in apple orchard under a high density of caterpillars (Mols and Visser, 2007). These studies prove that offering nest boxes can have a positive effect on bird population allowing a better control of pests, even in cases of outbreaks.

7. References

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APPENDIX

Table 1. List of detected birds species during the field work included in the analysis, common and scientific names (taxonomic order) and their diets according to to the Elton traits database.

Common name	Scientific name	Diet
Starling	*Sturnus*	Omnivore
Great spotted woodpecker	*Dendrocopos major*	Omnivore
European green woodpecker	*Picus viridis*	Invertebrate
Common chiffchaff	*Phylloscopus collybita*	Invertebrate
Common chaffinch	*Fringilla coelebs*	Invertebrate
Common blackbird	*Turdus merula*	Omnivore
European goldfinch	*Carduelis carduelis*	Plant seed with 10% Invertebrate
House sparrow	*Passer domesticus*	Omnivore
European robin	*Erithacus rubecula*	Omnivore
Great tit	*Parus major*	Omnivore
Long-tailed tit	*Aegithalos caudatus*	Invertebrate
Eurasian blue tit	*Cyanistes caeruleus*	Omnivore
Crested tit	*Lophophanes cristatus*	Invertebrate
Carrion crow	*Corvus corone*	VertFishScav with 30% Invertebrate
Meadow pipit	*Anthus pratensis*	Invertebrate
Cirl Bunting	*Emberiza cirlus*	Plant seed with 30% Invertebrate
Corn bunting	*Emberiza calandra*	Plant seed with 30% Invertebrate
Eurasian collared dove	*Streptopelia decaocto*	Plant seed with 10% Invertebrate
Eurasian nuthatch	*Sitta europaea*	Invertebrate
Short-toed treecreeper	*Certhia brachydactyla*	Invertebrate
Woodlark	*Lullula arborea*	Omnivore
Eurasian blackcap	*Sylvia atricapilla*	Omnivore
Sardinian warbler	*Sylvia melanocephala*	Omnivore
Dartford warbler	*Sylvia undata*	Invertebrate
Zitting cisticola	*Cisticola juncidis*	Invertebrate
Black-shouldered kite	*Elanus caeruleus*	VertFishScav with 20% Invertebrate
White wagtail	*Motacilla alba*	Invertebrate
European stonechat	*Saxicola rubicola*	Invertebrate
Eurasian wren	*Troglodytes troglodytes*	Invertebrate
Eurasian jay	*Garrulus glandarius*	Omnivore
Iberian magpie	*Cyanopica cooki*	Invertebrate
Eurasian magpie	*Pica pica*	VertFishScav with 20% Invertebrate
Goldcrest	*Regulus regulus*	Invertebrate
Iberian gray shrike	*Lanius meridionalis*	Invertebrate
Red-legged partridge	*Alectoris rufa*	Plant seed with 10% Invertebrate
Booted eagle	*Hieraaetus pennatus*	VertFishScav with 10% Invertebrate
European bee-eater	*Merops apiaster*	Invertebrate
Common redstart	*Phoenicurus phoenicurus*	Invertebrate
Common swift	*Apus apus*	Invertebrate
Melodious warbler	*Hippolais polyglotta*	Invertebrate
Woodchat shrike	*Lanius senator*	Invertebrate

Table 2. Summary of generalized linear models relating presence-absence of two bird species (Sardinian warbler and European nuthatch) during with distance to the road, road identification (A13, N10, N119) and observers and generalized linear models relating presence-absence of European stonechat during both seasons with distance to the road, road identification (A13, N10, N119) and observers. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600 m, respectively.

	P/A Sardinian warbler				**P/A European nuthatch**				**P/A European stonechat winter**				**P/A European stonechat spring**			
Predictors	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*
(Intercept)	-0.35	0.94	-0.37	0.709	-4.14	1.10	-3.77	**<0.001**	-1.49	1.02	-1.45	0.147	0.92	0.91	1.01	0.314
PC [PC 300m]	1.14	0.64	1.79	0.074	1.48	0.68	2.15	**0.031**	-0.42	0.65	-0.64	0.520				
PC [PC 600m]	1.93	0.72	2.68	**0.007**	1.57	0.69	2.26	**0.024**	-1.72	0.87	-1.99	**0.047**	-3.29	1.25	-2.63	**0.009**
Road [N10]	-1.40	0.83	-1.68	0.092	0.13	0.82	0.16	0.872	-0.68	0.92	-0.75	0.456				
Road [N119]	-0.03	0.84	-0.04	0.971	-0.02	0.82	-0.03	0.977	-1.41	0.98	-1.44	0.151	1.33	1.25	1.06	0.288
Observers	0.48	0.62	0.77	0.440	1.99	0.64	3.13	**0.002**	1.06	0.77	1.38	0.167				
Observations	75				75				75				22			
R^2 Tjur	0.177				0.245				0.102				0.437			

Table 3. Summary of generalized linear models relating presence-absence of three bird species (European goldfinch, Woodlark, Corn bunting) during winter with distance to the road, road identification (A13, N10, N119) and observers. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600 m, respectively.

	P/A European goldfinch				**P/A Woodlark**				**P/A Corn bunting**			
Predictors	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*
(Intercept)	-3.24	1.57	-2.06	**0.039**	-3.67	1.50	-2.45	**0.014**	0.48	1.24	0.39	0.700
PC [PC 300m]	2.04	1.13	1.81	$\boxed{0.070}$	2.07	1.13	1.83	$\boxed{0.068}$	-0.67	0.83	-0.80	0.423
PC [PC 600m]	0.01	1.45	0.01	0.993	1.56	1.17	1.34	0.181	-1.93	1.17	-1.65	$\boxed{0.099}$
Road [N10]	-0.76	1.24	-0.61	0.540	-0.73	1.17	-0.62	0.534	-0.67	0.96	-0.69	0.487
Road [N119]	-0.46	1.19	-0.39	0.700	0.26	1.04	0.25	0.805	-1.47	1.08	-1.36	0.174
Observers	0.33	0.96	0.35	0.727	0.34	0.80	0.42	0.671	-0.78	0.99	-0.79	0.431
Observations	75				75				75			
R^2 Tjur	0.095				0.088				0.138			

Table 4. Summary of generalized linear models relating presence-absence of five bird species (Common chaffinch, European robin, Sturnus, Short-toed treecreeper and Great tit) with distance to the road, road identification (A13, N10, N119) and observers. PC 30m, PC 300m and PC 600m stand for sampling points at 30, 300 and 600 m, respectively.

	P/A Common chaffinch Spring				**P/A European robin**				**P/A Sturnus**				**P/A Short-toed treecreeper**				**Great tit**			
Predictors	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*	*Estimate*	*std. Error*	*Statistic*	*p-value*
(Intercept)	-0.77	0.87	-0.89	0.375	-0.42	0.89	-0.47	0.637	-4.31	1.34	-3.21	**0.001**	-2.45	1.08	-2.27	**0.023**	0.64	1.10	0.58	0.563
PC [PC 600m]	1.55	1.10	1.41	0.157	-0.75	0.65	-1.16	0.248	1.01	0.71	1.42	0.156	0.58	0.76	0.77	0.444	0.19	0.72	0.26	0.793
Road [N119]	1.81	1.09	1.66	0.096	-0.98	0.75	-1.30	0.194	1.64	1.18	1.39	0.166	-1.77	0.99	-1.79	0.073	0.33	0.83	0.40	0.687
PC [PC 300m]					-0.83	0.66	-1.26	0.206	0.51	0.72	0.71	0.479	0.79	0.74	1.07	0.286	-1.13	0.92	-1.23	0.220
Road [N10]					-2.16	0.81	-2.66	**0.008**	0.70	1.23	0.57	0.572	0.01	0.84	0.01	0.992	-0.92	0.99	-0.93	0.352
Observers					1.67	0.60	2.77	**0.006**	1.10	0.63	1.73	0.083	0.95	0.73	1.30	0.194	-1.42	0.81	-1.76	0.079
Observations	22				75				75				75				75			
R^2 Tjur	0.206				0.198				0.164				0.133				0.110			